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EVALUATION OF RHIZOBIAL ISOLATES FROM *ALBIZIA LEBBECK* FOR TEMPERATURE TOLERANCE

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ABSTRACT

A total of 40 rhizobial isolates capable of nodulating *Albizia lebbeck* (L.) Benth. were obtained from thirteen locations in Pakistan with dominant communities of *Albizia* plantation, and were evaluated for temperature tolerance under laboratory conditions. Rhizobial isolates were grown on yeast-extract mannitol agar medium and incubated at 28, 32, 36, and 40° C for seven days. Distinct variations in temperature tolerance were observed for isolates obtained from different locations. While all isolates grew at 28° C and 70% at 32° C, higher incubation temperatures sharply reduced the proportion of isolates able to grow. Only three isolates from Punjab and two from Sindh were able to grow at 40° C. The information on temperature tolerance by *A. lebbeck* root-nodule bacteria may be useful for the characterization of the strains. Selection of temperature-tolerant rhizobial strains may contribute greatly to the forest productivity under high temperature conditions and reforestation of degraded areas in the tropics.

KEY WORDS: tree legume, rhizobia, temperature tolerance, forest productivity

INTRODUCTION

Siris (*Albizia lebbeck* [L.] Benth.) is a well known, fast growing tree of Pakistan. It is widely grown in the plains up to an elevation of 1200 m, in farmlands, along roadsides, irrigated plantations, and riverine tracts. It grows on a variety of soils, but deep loamy soil is preferred. Its leaves contain 16.8-25.5% crude protein and are an excellent source of fodder (Quraishi, *et al.* 1993). *Albizia lebbeck* forms symbiotic associations with *Rhizobium*, and can fulfill its own nitrogen requirements. This symbiotic relationship also enriches the soil with nitrogen for succeeding crops. Moreover, it is a salt-tolerant plant and it can be cultivated in salt affected soil (Prinsen 1986).

Nitrogen-fixing efficiency of legumes is greatly altered by the environmental impacts on both host and symbiont (Barnet, *et al.* 1988; Bordeleau & Prevost 1994). Most studies on symbiotic performance of rhizobia under environmental stress have concentrated on herbaceous legumes within an agricultural context (Athar & Johnson 1996, 1997). Our knowledge about the constraints affecting *Rhizobium* symbiosis in woody legumes is very limited. Temperature can affect rhizobial persistence in inoculants during shipment or in storage, can influence survival in soil, and can limit both nodulation and nitrogen fixation (Graham 1992). High root temperatures will also delay nodulation or restrict it to the subsurface region, where temperatures are not as extreme. It has been shown for various plants grown in tropical and subtropical regions, that root temperatures in the range of 35° C to 40° C are detrimental to nodule formation and nitrogen fixation (Arayangkoon, *et al.* 1990; Hungria & Franco 1993; Hungria, *et al.* 1993; Kishinevsky, *et al.* 1992; La Favre & Eaglesham 1986; Michiels, *et al.* 1994). Effect of root temperature on nodulation and fixation by legumes is modified by strain of rhizobia (Arayangkoon, *et al.* 1990; Kishinevsky, *et al.* 1992; La Favre & Eaglesham 1986).

The productivity of legume trees depends upon their nitrogen-fixing potential in unfavorable soil conditions. Temperature, in conjunction with drought, is a serious problem, particularly in Pakistan (Athar & Johnson 1996). A detailed understanding of various environmental constraints on nitrogen-fixing potential of woody legumes is necessary to increase and obtain sustainable yields from forest plantations. The rhizobia isolated from *Acacia nilotica* (L.) Del. showed differences in survival at elevated temperatures (Athar 1994). The effect of temperature on rhizobia from other woody legumes of Pakistan is not known. The objective of the present investigation was to determine the effect of temperature on growth of indigenous rhizobia isolated from *Albizia lebbeck*.

MATERIALS AND METHODS

Nodulation in *Albizia lebbeck* was examined from various forest plantations of Pakistan with dominant communities of *Albizia* vegetation. The roots of young plants growing close to or under the canopy of adult plants were excavated and observed for nodulation. Details of the procedure for the retrieval of nodules from legume trees are provided elsewhere (Athar 1994). Some nodules were collected from the seedlings raised in plastic bags containing natural soil. Nodules selected randomly from each site were stored in small vials over silica gel for later isolation of root-nodule bacteria by the method of Somasegaran & Hoben (1994). A total of 40 isolates of rhizobia (Table 1) were obtained and maintained on yeast-extract mannitol agar slants at 5° C.

Table 1. Site, location, and elevation for rhizobial strains from *Albizia lebbeck*.

Province/Site	Location	Elevation (m)	Strains
Punjab			
Changa Manga	31° 50′ N, 73° 58′ E	190	AL 10, AL 11, AL 12, AL 13, AL 14, AL 15
Jhang	31° 34′ N, 72° 30′ E	170	AL 16, AL 17
Chichawatni	30° 32′ N, 72° 42′ E	160	AL 18, AL 19, AL 20, AL 21, AL 22
Khanewal	30° 18′ N, 71° 56′ E	140	AL 23, AL 24, AL 25, AL 26
Dera Ghazi Khan	29° 50′ N, 70° 15′ E	150	AL 27, AL 28, AL 29
Cholistan	28° 15′ N, 70° 45′ E	120	AL 30, AL 31, AL 32, AL 33
Sindh			
Shikarpur	27° 57′ N, 68° 38′ E	58	AL 34, AL 35
Khairpur	27° 32′ N, 68° 46′ E	62	AL 36, AL 37
Hyderabad	25° 22′ N, 68° 22′ E	27	AL 38, AL 39, AL 40
Tando Bago	24° 47′ N, 68° 58′ E	14	AL 41, AL 42
N.W.F.P.			
Mardan	34° 90′ N, 71° 46′ E	329	AL 43, AL 44
Kohat	33° 34′ N, 71° 27′ E	492	AL 45, AL 46
Dera Ismail Khan	31° 54′ N, 70° 54′ E	172	AL 47, AL 48, AL 49

AL designation indicates that the rhizobial strain is from *Albizia lebbeck*.

All isolates were screened by Gram's staining and only those conforming to the description for the rhizobia (Somasegaran & Hoben 1994) were included for future testing.

Pure cultures of rhizobia were grown in yeast-extract mannitol broth for seven days at 28° C, diluted to 10^8 - 10^9 cells mL^{-1} with sterile distilled water and 0.1 mL was streaked on yeast-extract mannitol agar plates (Somasegaran & Hoben 1994). The plates were replicated four times for each isolate and were incubated at 28, 32, 36, and 40° C for seven days. Growth was detected by visual inspection as positive (visible growth) or negative (no growth).

Table 2. Temperature tolerance by *Albizia lebbeck* root-nodule bacteria.

Province	Total isolates	Temperature (° C)			
		28	32	36	40
Punjab	24	24 (100)	17 (71)	11 (46)	3 (13)
Sindh	9	9 (100)	7 (78)	4 (44)	2 (22)
N.W.F.P.	7	7 (100)	4 (57)	0 (00)	0 (00)
Total	40	40 (100)	28 (70)	15 (45)	5 (13)

Values in parentheses are the percentages of tolerant isolates.

RESULTS AND DISCUSSION

Root nodules of *Albizia lebbeck* were observed at all sampling locations, indicating wide distribution of rhizobia in Pakistani soil. These rhizobia varied in their response to temperature tolerance (Table 2). All 40 isolates grew at 28° C. However, elevated incubation temperatures markedly reduced the proportion of isolates to grow. About 70% of isolates tolerated a temperature of 32° C and 15% of isolates showed growth at 36° C. The rhizobial isolates from *A. lebbeck* showed marked geographic localization. While none of the isolates from North Western Frontier Province (N.W.F.P.) could grow at temperatures of 36 and 40° C, three isolates from Punjab and two from Sindh were able to grow at high temperatures (Table 2). High temperature is known to influence the growth and survival of rhizobia. Differences among strains in tolerance to high temperatures have been shown for *Bradyrhizobium japonicum* (Boonkerd & Weaver 1982; Hartal & Alexander 1984; Kennedy & Wollum 1988; Kishinevsky, *et al.* 1992), *Rhizobium meliloti* (Toro & Olivares 1986), *R. leguminosarum* (Moawad & Beck 1991), and *R. tropici* (Martinez-Romero, *et al.* 1991).

More recently, various rhizobial strains have been characterized by their ability to grow under elevated temperatures. Athar (1993, 1994) examined temperature tolerance of *Lens culinaris* and *Acacia nilotica* rhizobia from Pakistan and observed clear differences in survival of indigenous rhizobia at temperature as high as 36 and 40° C. Michiels, *et al.* (1994) also indicated differences in cell viability of bean-nodulating *Rhizobium* strains to thermal stress. In the tropics, soil temperatures between 40 and 60° C are not uncommon. Some rhizobial strains from tree legumes can nodulate and fix nitrogen at temperatures as high as 40° C and could represent a genetic source for nodulation at high temperatures with other species (Hungria, *et al.*

1993). *Albizia lebbeck* is a drought resistant tree, and its associated rhizobia are exposed to very dry and hot temperature conditions. In summer, temperatures in some parts of Punjab and Sindh exceed 40° C. Thus, soil temperature may influence natural populations of root-nodule bacteria. However, it has been shown that some rhizobial strains isolated from tropical tree legumes were able to fix nitrogen at extremely high temperatures (Hungria, *et al.* 1993). The development of a methodology for selecting temperature-tolerant rhizobial strains may contribute greatly to the success of nitrogen-fixing symbiosis under high temperature conditions. Additional research is required to elucidate the genetics and physiology of these elite strains before including them as inoculants in the reforestation of degraded areas in the tropics.

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